Application Note

Channels: TRPV1
Cells: Bilayers
Tools: Orbit Mini, Temperature Control

Temperature activation of TRPV1 reconstituted into planar lipid bilayers and recorded using the Orbit mini platform

The electrophysiology team at Nanion Technologies GmbH, Munich.

Summary

The transient receptor potential cation channel, subfamily V, member 1 (TRPV1), is a ligand-gated, non-selective cation channel widely expressed in the peripheral and central nervous system. The TRPV1 channel can be activated by a number of physical and chemical stimuli, including capsaicin (the active ingredient in chili peppers), noxious heat (typically >42˚C) and low pH. The TRPV1 channel is putatively involved in the perception and transmission of painful stimuli. Importantly, this channel is proposed to underlie many chronic pain states including inflammation, neuropathic pain and cancer pain, amongst others (1). These types of pain states are currently poorly managed by the pain medications available and this has led the pharmaceutical industry to seek novel targets for pain management, such as TRPV1. However, TRPV1 antagonists have so far failed in clinical trials due to an undesirable increase in core body temperature (2) resulting in hyperthermia. From these studies, it is proposed that tonically active TRPV1 channels are involved in maintaining normal body temperature and this could have significant influences on drug design. Finding novel compounds with differing effects on capsaicin activation and heat activation may be crucial in the discovery of lead compounds for the treatment of pain and other disease states.

Here we present data collected on the 4-channel Orbit mini with temperature control showing the potential use of the Orbit mini to record heat activation of single channel TRPV1.

Results

Formation of planar lipid bilayers on the MECA4

The bilayers are painted over the 4 wells of the MECA4 chip using either a hair brush or a glass rod. The lipid used in this study was DPhPC (1 mg/ml) dissolved in Octane. The brush or the glass rod was dipped into the lipids before painting over the wells.

Once the bilayers were formed, the purified protein TRPV1 was added to the bath solution for the reconstitution into bilayers. The activity of TRPV1 was checked using the agonist, Capsaicin (fig.1). The capsaicin was washed away before starting applying elevated temperature.

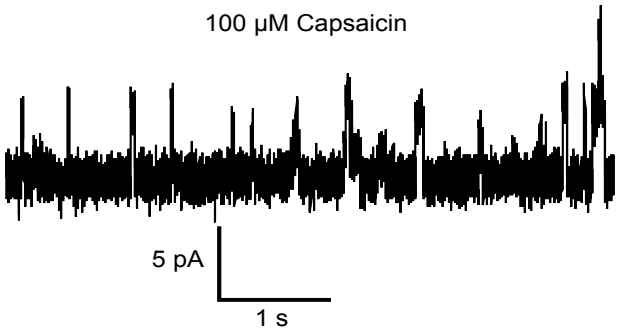

Figure 1. Current traces of reconstituted TRPV1 in DPhPC bilayers recorded at a transmembrane voltage of Vm= +50 mV. TRPV1 is activated using 100 µM Capsaicin (at room temperature). The recordings were done in symmetrical 200 mM KCl, 10 mM HEPES, pH 7.2 (with 20 kHz sampling rate and 10 kHz bandwidth).

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The temperature was raised progressively from Room temperature (~25 degrees Celsius) to 60 degrees Celsius. The figure 2 shows the current traces of bilayers with TRPV1 reconstituted at different temperatures.

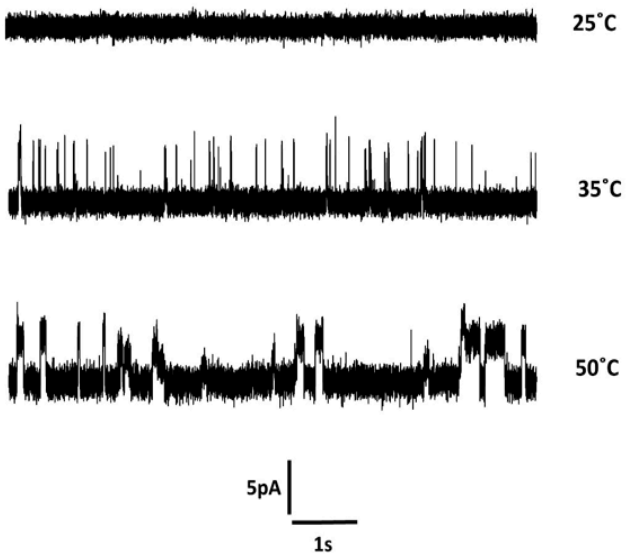


Figure 2. Current traces of reconstituted TRPV1 in DPhPC bilayers recorded at a transmembrane voltage of Vm= +50 mV. The activity of TRPV1 is temperature dependent. The activity of TRPV1 at 3 different temperatures around the activation threshold (>42 ˚C), 25, 35 and 50 ˚C is shown. The recordings were done in symmetrical 200 mM KCl, 10 mM HEPES, pH 7.2.

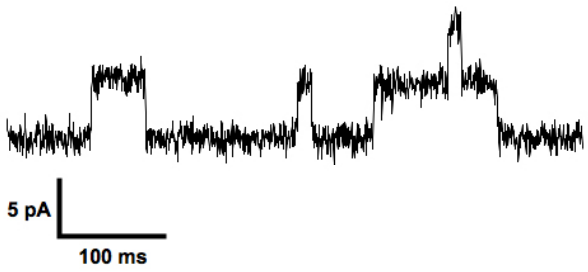
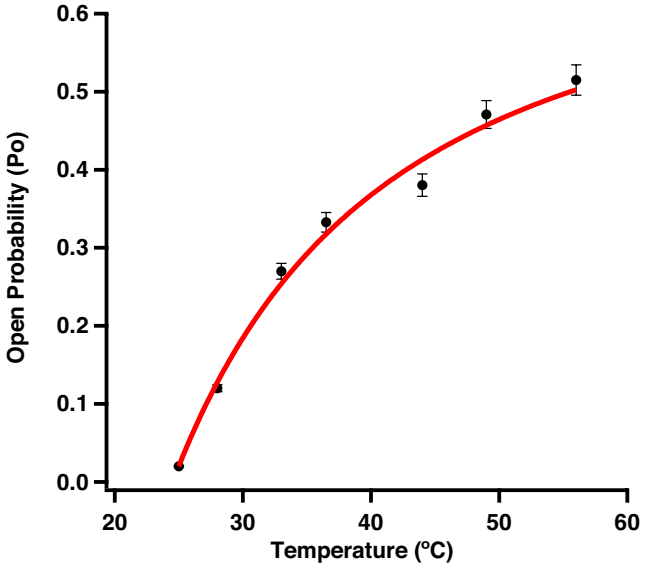


Figure 3. Current trace Zoomed in of reconstituted TRPV1 in DPhPC bilayers recorded at a transmembrane voltage of Vm= +50 mV.



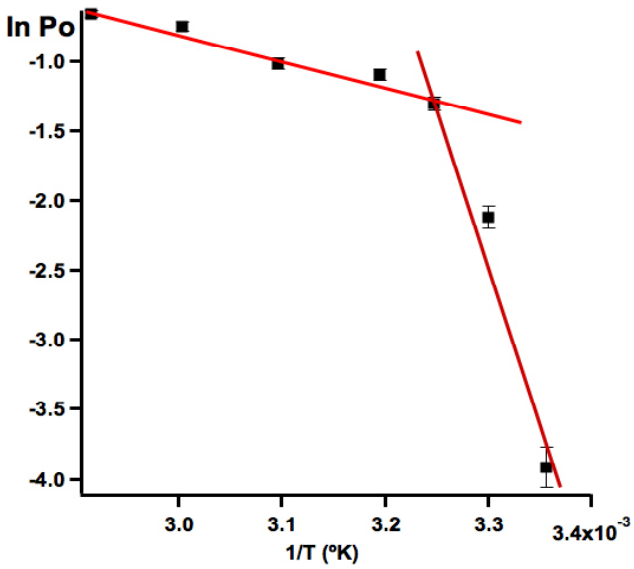


Figure 4. Effect of temperature on TRPV1 activity. Top, the open probability (P_o) versus the temperature and below, the Arrhenius plot of the same data resulting in a Q_{10} of 72

The conductance was derived from all point histograms and was found to be similar to previously published data on single channel conductance of TRPV1 in potassium solution (1). The conductance increased slightly with raising temperature (figure 4) as expected.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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The open probability was determined for each temperature together with the conductance of the channel opening. Figure 3 shows the open probability (P_o) versus the temperature. This graph allows us to show the Arrhenius plots (right) of the same data with P_o values as the natural logarithm (ln) and temperature (T) as reciprocal Kelvin. All single channel currents were recorded in a symmetrical K^+ solution. The open probability of TRPV1 increases with increasing temperature as shown in figure 3 (left). Bilayers without proteins did not respond to varying temperatures. P_o values were obtained for temperatures between 25 and 60 ˚C and were used to calculate the Q_{10} values of **72** from the Arrhenius plot of TRPV1. The heat activation of TRPV1 was reversible. The active cooling of the temperature control of the Orbit mini allowed us to cool down rapidly from 60 to 20 ˚C.

In conclusion, we have demonstrated stable recordings of the TRPV1 channel on our Orbit mini platform. 4 channels in parallel were recorded giving better statistics of the TRPV1 activity. The temperature range from 20 to 60˚C was applied using our Temperature Control system connected to the Orbit mini.

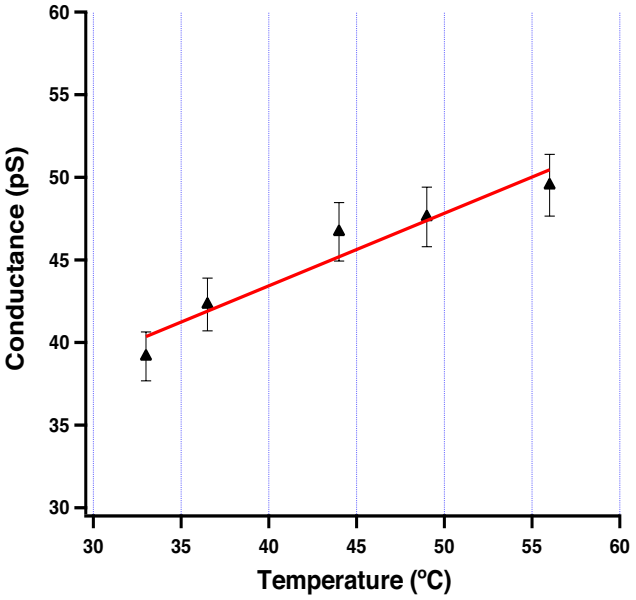

Figure 5. Temperature dependance conductance of TRPV1. The conductance increase slightly due to the raise of the temperature. Above 60 ˚C, the conductance was not calculated due to possible evaporation.

Methods

Bilayer formation and recordings

Symmetrical solutions were used: 200 mM KCl, 10 mM HEPES, pH 7.2.

The Orbit mini has an integrated 4 channel amplifier (eFOUR from Elements, Cesena, Italy) and integrate the MECA4 chip, multi-electrode-cavity-array chips (IONERA, Freiburg, Germany). Bilayers are formed by painting 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) dissolved in octane (1 mg/ml) over the 50 µm diameters wells of the MECA4. Recordings were done at 10 kHz bandwidth and 20 kHz sampling rate.

The purified protein, TRPV1 in detergent (DDM, 0.1%), with a concentration of 150 ng/ml, was added to the bath solution after the formation of the bilayers at an applied potential of +50 mV.

References

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2. Gavva, N.R., Treanor, J.J, Garami, A., Fang, L., Surapaneni, S., Akrami, A., Alvarez, F., Bak, A., Darling, M., Gore, A., Jang, G.R., Kesslak, J.P., Ni, L., Norman, M.H., Palluconi, G., Rose, M.J., Salfi, M., Tan, E., Romanovsky, A.A., Banfield, C., Davar, G. 2008. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain. 136: 202-210.

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Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

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fax +49 89 218997960
www.nanion.de • info@nanion.de